

IgG and IgE antibody responses to dust mite allergens among children with asthma in Brazil

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We compared exposure and levels of IgE and IgG antibodies to inhalant allergens in 20 children with asthma and positive skin tests to Dermatophagoides pteronyssinus, living in São Paulo, to 20 control children without history of asthma or allergy and negative skin tests to D. pteronyssinus, D. farinae, and Blomia tropicalis. In 16/20 and 17/20 houses of asthmatic and control children, respectively, at least one sample was obtained which contained >10 µg Der p I/g of dust. Serum IgE antibodies to D. pteronyssinus and Blomia tropicalis were >200 RAST U/mL in 19/20 and in 16/20 asthmatic children, respectively. In the control group, IgE antibodies to either mite species were <40 RAST U/mL in most cases. IgG anti-Der p I and anti-Der f I antibodies were detected in 17/20 asthmatics, as opposed to 3/20 controls. Neither cat nor cockroach allergens caused significant sensitization among asthmatic or control children. Although exposure to high levels of mite allergens was common in São Paulo, significant IgE and IgG antibody responses were detected only in children with asthma.

INTRODUCTION

In 1964, Voorhorst and Spielsma reported that mites of the family Pyroglyphidae (Dermatophagoides spp) were the major source of allergens in house dust.¹ Since then, the role of mites of the genus Dermatophagoides in causing allergic diseases, and asthma in particular, has been firmly established.^{2,3} Dermatophagoides spp. are undoubtedly the predominant mite species in many parts of the world. Methods to quantitate accurately Dermatophagoides major allergens Der p I, Der f I and Group II in house dust are available, and have been fundamental for assessing the rôle of exposure to mite allergens in sensitization and development of allergic diseases.⁴⁻¹²

In tropical countries, other mite

species have been reported to be also highly prevalent, particularly *Blomia tropicalis*.¹³⁻¹⁸ Previous studies have shown that *D. pteronyssinus*, *B. tropicalis* and *B. kulagini* are important causes of sensitization among asthmatic patients in Brazil.¹⁶⁻¹⁸ In addition, results of both immunoabsorption experiments and RAST inhibition demonstrated that *Blomia* spp. have species-specific allergens as well as crossreactive antigens with *D. pteronyssinus*.^{16,17} Preliminary analysis of allergen levels in houses of asthmatic children living in São Paulo, Brazil has been performed. Strikingly, levels of >10 µg Der p I/g of dust were found in 90% of the houses, while cat and cockroach allergens were undetectable or found in very low levels.¹⁶

The aim of the present study was to quantitate indoor allergen levels and antibody responses to those allergens in a group of mite allergic asthmatic children, and to compare both exposure and sensitization to a group of nonasthmatic nonatopic children from the same area. No significant differences in exposure to mite allergens were observed between asthmatic and control children. However, only children with

asthma had high levels of both IgE-specific and IgG-specific antibodies to mite allergens.

SUBJECTS AND METHODS

Subjects

A total of 40 children were enrolled in the present study. Twenty of the children, aged 6 to 16 years, were atopic asthmatic patients selected from patients who attended the Pediatric Allergy Clinic at Paulista School of Medicine, São Paulo. All the asthmatic children had positive immediate skin tests to *D. pteronyssinus*. In addition, 19/20 and 18/20 asthmatics also had positive immediate skin tests to *D. farinae* and *B. tropicalis* extracts, respectively. Twenty control children, aged 7 to 18 years, were selected from the Pediatric Emergency Room of the same institution, with no personal history of allergic disease and no respiratory symptoms at the occasion of enrollment. Control children had negative immediate skin tests to all three mite extracts. A positive skin test was defined by a wheal size >5 x 5 mm in diameter on prick test. Serum samples were obtained from each child, and they had their houses visited for collection of dust

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samples. Ten of the asthmatic children had participated in a previous study.¹⁶ Sera and house dust samples were again obtained from these patients a year after the original study¹⁶ and this second set of samples was included in the present study.

D. pteronyssinus, *D. farinae*, and *B. tropicalis* Extracts

Extracts made from isolated *D. pteronyssinus* and *D. farinae* bodies were obtained from Hollister-Stier, Spokane, WA. Both whole culture and mite body *B. tropicalis* extracts were prepared as described previously.¹⁶

Total IgE and IgE Antibodies to Mite, Cat, and Cockroach

Total serum IgE was measured by ELISA, modified from previously described RIA.^{19,20} Results were expressed as International Units per mL (1 IU/mL = 2.4 ng IgE/mL). IgE antibodies to *D. pteronyssinus*, *D. farinae*, *B. tropicalis*, cat, and cockroach were measured by radioallergosorbent test (RAST).¹⁹ Each assay for IgE antibody was quantitated on the basis of a control curve constructed using *D. farinae* discs and serial 2-fold dilutions of a mite allergic serum pool (UVA 87/01) which has been substandardized against an international reference serum pool (NIBSC Code no. 82/528). The UVA serum pool contained 1,000 RAST units/mL of specific IgE antibodies to *D. farinae*. It has been estimated that one RAST unit is equivalent to 0.1 ng of IgE.¹⁹

IgG antibodies to Der p I and Der f I

An antigen-binding RIA was used to measure serum IgG antibodies to Der p I and Der f I, as described previously.^{21,22}

Allergen Levels in House Dust

House dust samples were obtained from four sites of each subject's house: bedding, bedroom floor, TV room and kitchen, using a handheld vacuum cleaner. Floor dust was collected by vacuuming an area of 1

m² for two minutes, or two minutes for bedding.²³ Fine dust was obtained by sieving through a 0.3-mm mesh screen. Group I major *D. pteronyssinus* and *D. farinae* allergens, Der p I and Der f I; Fel d I, the major cat allergen from *Felis domesticus*; and Bla g I, a crossreactive cockroach allergen purified from *Blattella germanica* were measured by monoclonal antibody based ELISA, as described elsewhere.^{7,24,25} Group II *Dermatophagoides* sp. allergens were quantitated using a solid phase monoclonal antibody based RIA.²⁶ In each assay, reference preparations containing known levels of Der p I, Der f I, Fel d I, and group II allergens were used to make a control curve, and results were expressed as µg/g of dust. Cockroach assays were quantitated based on a reference German cockroach extract (UVA 89/01), and results were expressed as arbitrary units of Bla g I/g of dust.

Statistical Analysis

Statistical analysis was performed

using linear regression and Mann Whitney test, and *P* values <.05 were considered as significant.

RESULTS

Antibody Responses to Inhalant Allergens

IgE antibodies to *D. pteronyssinus*, *D. farinae*, and *B. tropicalis* were >200 RAST U/mL in 19/20, 16/20, and 16/20 asthmatic children, respectively. The geometric means were 1,549, 447, and 794 RAST units/mL. Specific IgE antibodies to mites were usually undetectable in the control group, and none had specific IgE >200 RAST U/mL to any of the mite species (Fig 1). IgE antibodies to cat and cockroach were undetectable or found in very low levels in most subjects. Only one patient had significant IgE antibody to cat, and only two patients had significant IgE antibody to cockroach (Fig 1). These results confirm our previous studies, and show that mites are a major cause of sensitization among asthmatic

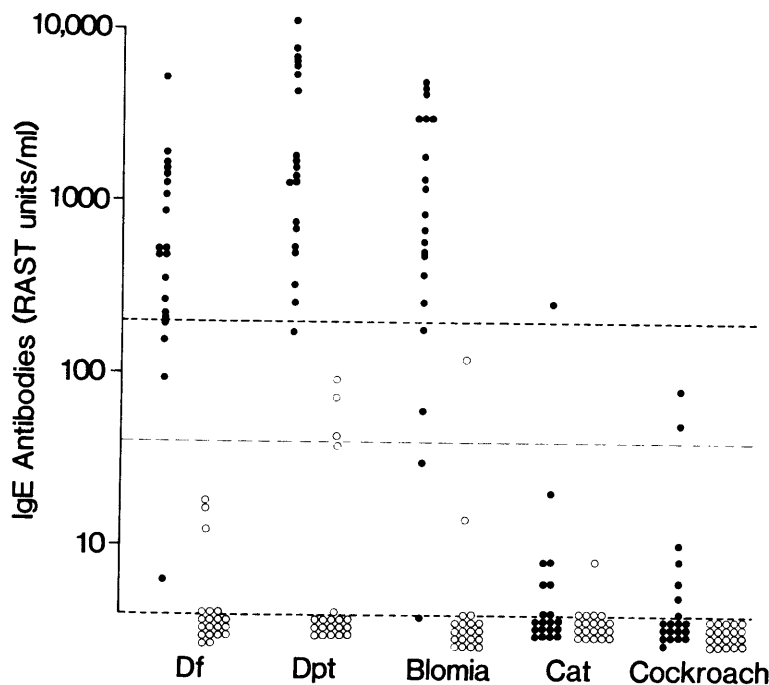


Figure 1. Measurements of IgE antibodies to *D. farinae* (Df), *D. pteronyssinus* (Dpt), *B. tropicalis* (Blomia), cat, and cockroach in sera from asthmatic children (●) and controls (○). The lower dashed line represents the detection level of the assay (4 RAST units/mL). The upper dashed lines represent 40 and 200 RAST U/mL, respectively.

children in Brazil. Total serum IgE was significantly higher in asthmatic patients as compared with control children (geometric mean G.M. 1,318 IU/mL and 212 IU/mL, respectively, $P < .05$). There was a good correlation between total IgE and specific IgE to mite, cat, or cockroach. The majority of asthmatic patients (16/20) had elevated total IgE (>400 IU/mL), and the highest levels of specific IgE (>200 RAST U/mL) were found in this group. IgE antibodies to *D. pteronyssinus* accounted for the highest values, indicating that *D. pteronyssinus* was the predominant allergen (Fig 2, A and B). In the control group, the majority of individuals had undetectable or low specific IgE (<40 RAST U/mL), and their total IgE was lower. In this group, only 7/20 had IgE >400 U/mL.

IgG antibodies to *Der p* I and *Der f* I were present in 18/20 and 17/20 sera from patients with asthma, respectively. Only 3/20 controls had detectable IgG anti-*Der p* I antibodies, and one of these had a positive RAST to *D. pteronyssinus* (42 U/mL) (Fig 3). When analyzing sera from both groups of patients, there was a significant correlation between IgE antibodies to *D. pteronyssinus* and *D. farinae* ($r = .57$, $P < .001$) (Fig 4A), and between IgG antibodies to *Der p* I and *Der f* I ($r = .51$, $P < .05$) (Fig 4B).

Indoor Allergen Exposure

Major allergens from dust mites, cat, and cockroach were measured in four sites from the houses of both patients with asthma and controls (160 samples in all). Mite allergens were by far the most prevalent allergens in both asthmatic patients' and

controls' homes. No significant differences were observed between levels of *Der p* I in dust samples from either group of children, and the highest levels of this allergen were measured in bedding samples (Table 1). Similar results were obtained for group II allergens. A good correlation was found between *Der p* I and group II allergen levels when all samples were analyzed ($r = .51$, $P < .05$) (Fig 5). Linear regression analysis showed that there was no significant quantitative correlation between exposure to *Der p* I and either levels of IgE antibodies to *D. pteronyssinus* or IgG antibodies to *Der p* I ($r = -.13$, $P = .58$; $r = -.14$, $P = .52$, respectively). In keeping with previous observations that *D. farinae* is not common in Brazil, *Der f* I levels were undetectable in most cases; in 8/160 dust samples

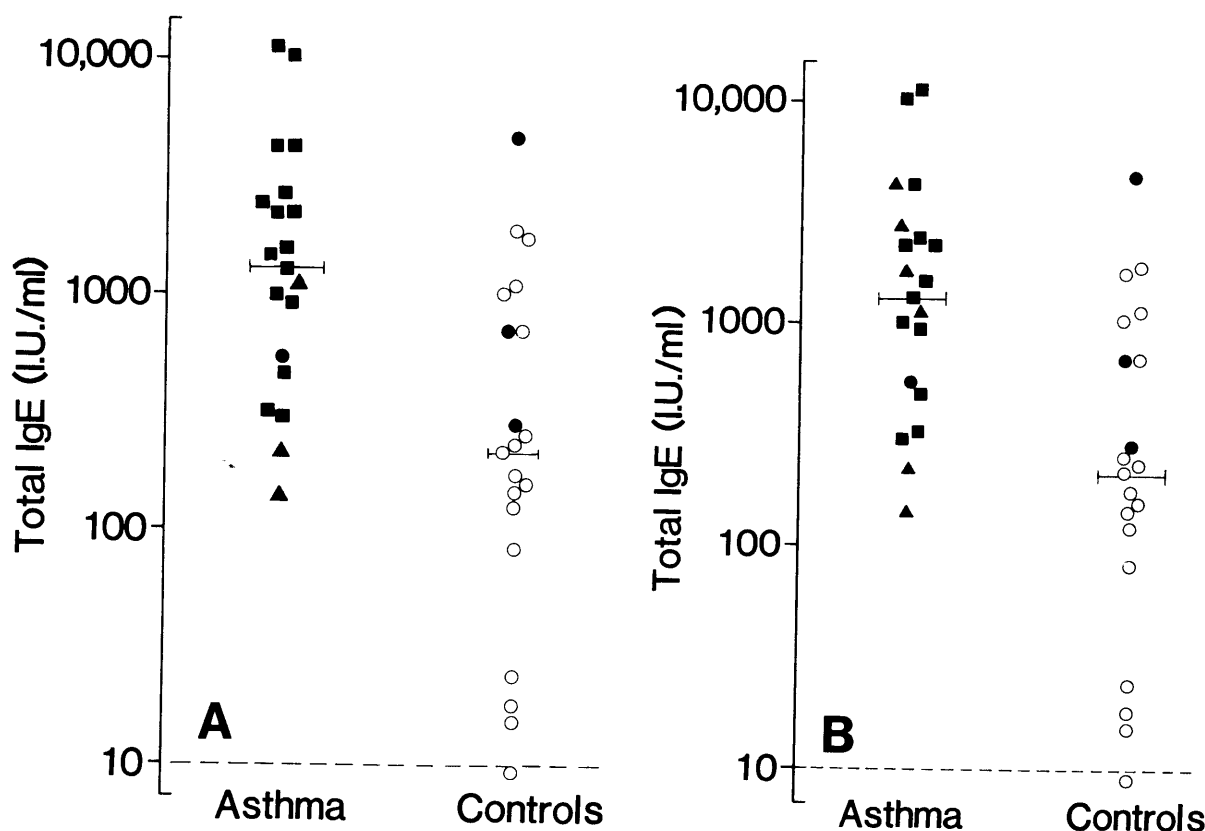


Figure 2. Total serum IgE levels in 20 patients with asthma and 20 controls. Sera were grouped according to the levels of IgE antibody, measured by RAST, to any of the five allergens studied (Fig 2A) or to the levels of IgE antibody to *D. pteronyssinus* (Fig 2B) as follows: ■ ≥ 800 U/mL; ▲ 200 to <800 U/mL; ● 40 to <200 U/mL; and ○ <40 U/mL. The geometric mean values for the two groups are shown by bars (GM for patients with asthma = 1,318 IU/mL, GM for controls 212 IU/mL).

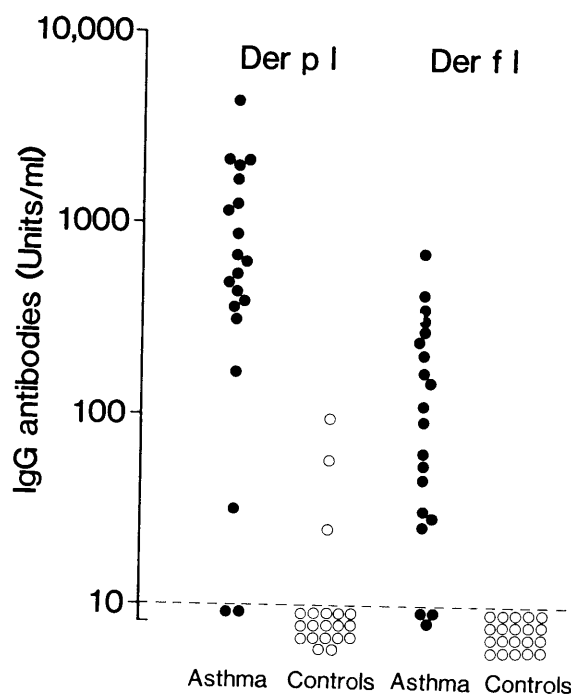


Figure 3. Quantitative measurements of IgG anti-*Der p I* and anti-*Der f I* antibodies in sera from patients with asthma (●) and controls (○), using antigen-binding RIA.

Der f I was $>2 \mu\text{g/g}$, and in only 1/40 houses *Der f I* level was $>10 \mu\text{g/g}$. *Der f I* levels ranged from 2 to 25 $\mu\text{g/g}$ of dust, while *Der p I* levels ranged from 4 to 210 $\mu\text{g/g}$ in the same samples. In all but one case, *Der f I* accounted for $<25\%$ of the total group I allergen. The presence of cat(s) was reported in one patient's house and three houses of controls, and in 3/4 houses *Fel d I* levels were $>8 \mu\text{g/g}$. One asthmatic patient had 250 RAST U/mL of IgE antibody to cat, and this patient reported a cat present within the house, with $>8 \mu\text{g}$ *Fel d I/g* in 3/4 of his dust samples. Exposure to cockroach allergen was <0.6 units *Bla g I/g* in all samples.

DISCUSSION

Results of the present study demonstrated that both asthmatic and control children were exposed to very high levels of mite allergens, and no significant difference was observed between levels in houses of asthmatic patients and controls, regarding exposure to *D. pteronyssinus* allergens (*Der p I* or *Der p II*).

These results agreed with previous reports from the United States and Europe^{8,9} and supported the view that not only exposure to high levels of dust mite allergens but also the genetic predisposition to make IgE antibody responses are essential for sensitization.

Exposure to high levels of mite allergen was found both in asthmatic and control children, however, only asthmatic patients developed a strong IgE and IgG antibody response to *Dermatophagoides* spp antigens. The issue of whether IgG antibody responses develop in exposed nonatopic individuals is controversial. Kemeny et al reported that the majority of both atopic and nonatopic individuals were capable of mounting an IgG1 antibody response to house dust mite and grass pollen allergens upon natural exposure.²⁷ On the other hand, Solimon and Rosenstreich demonstrated that only 10% to 11% of nonatopic subjects had either specific IgG or IgE antibodies to dust mite.²⁸ It is possible that the discrepancy between results from several studies are due

to different methods used for measuring specific IgG antibodies, which may reflect detection of low affinity antibodies or IgG antibodies to other antigens in the ELISA.^{27,28} In order to resolve this issue, it would be necessary to compare IgG antibody responses in large populations of nonatopic individuals from areas of high, low, or negligible exposure to mite allergens. Antigen binding RIA has consistently shown undetectable or very low levels of IgG antibodies to Group I major *Dermatophagoides* allergens in subjects with negative skin tests to *D. pteronyssinus*.²⁹ Previous prospective studies using antigen binding RIA have shown that a large group of skin test-negative children had IgG antibodies to *Der p I* at background levels over the first 5 years of life, whereas in the atopic children there was a progressive increase of IgG antibodies from the second year of life onwards.³⁰

A good correlation was found between IgE antibodies to *D. pteronyssinus* and *D. farinae*, and between IgG antibodies to *Der p I* and *Der f I*. Exposure among subjects in the present study was primarily to *D. pteronyssinus*, therefore we assumed that IgG and IgE antibodies to *D. farinae* reflect crossreactivity with *D. pteronyssinus* in most cases. Immunoabsorption experiments using sera from the US have shown that human IgE antibodies to *Der p I* and *Der f I* were largely directed against crossreactive epitopes, however, a small proportion of IgE induced by *Der f I* could be directed against species-specific determinants.³¹ These observations are supported by the fact that the amino acid sequence homology between *Der p I* and *Der f I* is high (81%).³² In the US, patients are exposed to both *D. pteronyssinus* and *D. farinae*, however, in our study levels of both IgE and IgG antibodies were on average 3- to 5-fold lower for *D. farinae* allergens (0.8 to 28- and 0.3 to 68-fold lower, respectively). Other studies in areas of predomi-

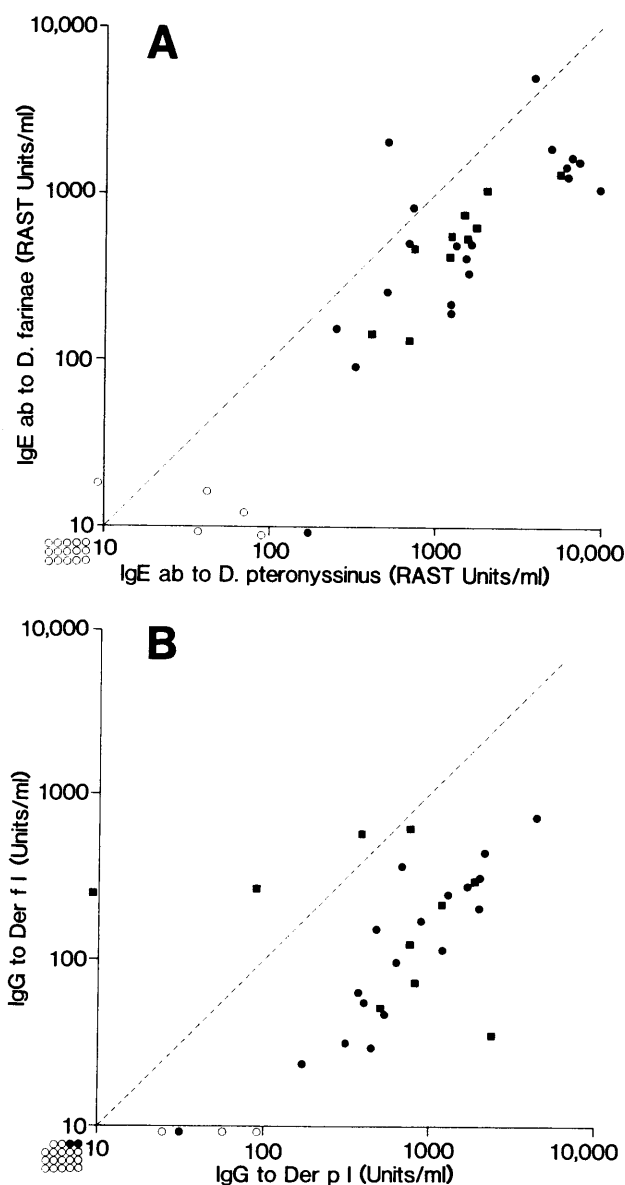


Figure 4. Linear regression analysis of the correlation between IgE antibodies to *D. pteronyssinus* and *D. farinae* (Fig 4A), and between IgG antibodies to Der p I and Der f I (Fig 4B) in sera from patients with asthma (●) and controls (○). Results on sera ($n = 10$) from asthmatic patients who participated in a previous study¹⁶ were also included in the analysis (■).

nant exposure to *D. pteronyssinus* (eg, Europe and Japan) have also suggested that human IgE antibodies recognize species-specific epitopes on Der p I, as well as cross-reactive determinants.^{33,34} Together these results suggest that upon heavy exposure to *D. pteronyssinus*, a larger amount of the IgG and IgE antibodies are directed against spe-

cies-specific epitopes and, in addition, that these antibodies may have a higher affinity for Der p I.

Although there was a significant difference between levels of total IgE in sera from asthmatic children as compared with controls, the mean IgE levels in our study were higher than those reported in studies on the epidemiology of asthma in the

United States.^{19,23} Our levels were comparable to a population of Egyptian asthmatic and nonasthmatic subjects studied for the prevalence of IgE antibodies to inhalant allergens and relationship to parasitic infection.³⁵ We believe those high levels of total IgE could be caused by infection with intestinal parasites (helminths) among some of the children in our study. The implications of the upregulation of IgE production by parasites to the development of IgE antibodies to inhalant allergens and asthma have not been clearly established.

Our results suggested that a major proportion of asthma in São Paulo could be directly attributable to continued exposure to high levels of mite allergens. At present, there are no methods for measuring exposure to *B. tropicalis* other than microscopic identification and counting of mites. The finding of high levels of anti-*B. tropicalis*-specific IgE antibodies in sera from our asthmatic patients and the existence of species-specific *B. tropicalis* allergens¹⁶ suggest that this mite species is also an important cause of sensitization in São Paulo.

In conclusion, in the present study we provide data to support the view that mite allergen exposure plays a major role in sensitization and development of asthma among children in São Paulo. High levels of mite allergens were also found in houses of non-atopic children from the same area. Recently, Sporik et al have shown that exposure to $>10 \mu\text{g}$ Der p I/g of dust in early childhood was an important determinant for the subsequent development of asthma by the age of 11 years old.³⁶ In addition, several studies have demonstrated decrease of bronchial hyperreactivity and improvement of symptoms following mite avoidance regimens.³⁷⁻³⁹ Our results suggest that mite allergen avoidance measures could have a major role in decreasing the rate of sensitization or perhaps the development of symptoms of asthma in genetically pre-

Table 1. Exposure to *Der p* I Among Patients with Asthma Compared with Controls

Site	Asthma <i>Der p</i> I ($\mu\text{g/g}$)* GM (95% confidence limits)	Highest Values	Controls <i>Der p</i> I ($\mu\text{g/g}$) GM (95% confidence limits)	Highest Values	P Value†
Bedding	22.2 (48.5; 10.2)	687.5	16.4 (31.2; 8.6)	306.2	N.S.
Bedroom floor	5.3 (9.6; 2.9)	136.9	3.3 (8.6; 1.3)	88.8	N.S.
TV room	4.2 (7.9; 2.3)	128.7	2.5 (5.7; 1.1)	95.0	N.S.
Kitchen	0.2 (0.7; 0.06)	6.2	0.08 (0.3; 0.02)	10.4	N.S.

* *Der p* I levels were measured by monoclonal antibody based ELISA.

† Levels of *Der p* I in asthmatics and controls were compared by Mann-Whitney test. P values < .05 were considered significant.

GM = geometric mean.

NS = not significant.

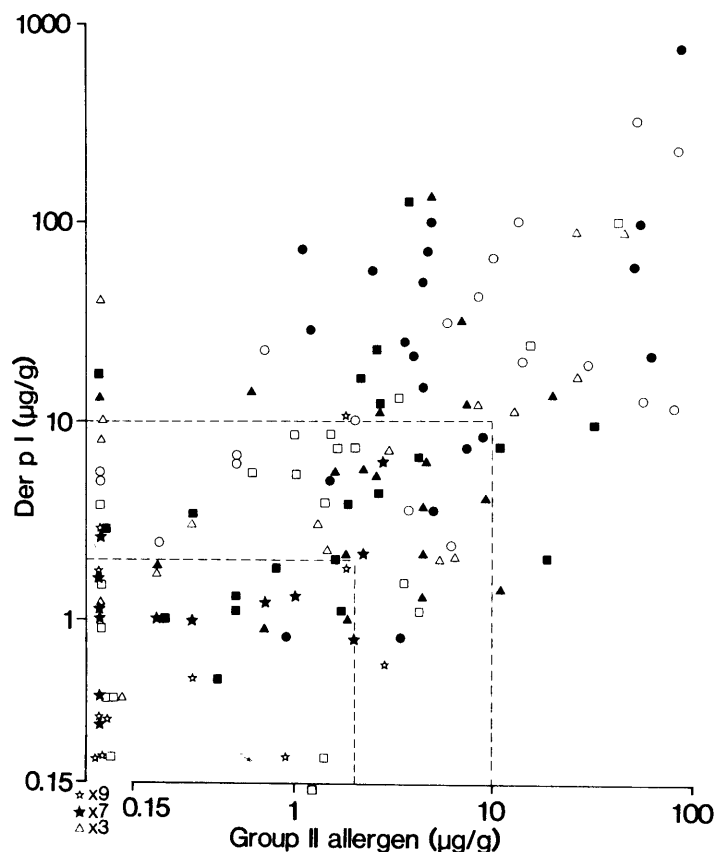


Figure 5. Levels of *Der p* I and group II allergens in 160 dust samples from houses of asthmatic patients (solid symbols) and controls (open symbols), collected from four sites of each subject's homes: bedding (●, ○); bedroom floor (■, □); TV room (▲, △); and kitchen (★, ☆). Dashed lines indicate 2 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ of dust, which have been proposed as threshold levels for sensitization and for development of acute symptoms of asthma in mite-allergic individuals, respectively.²

disposed children who are continuously exposed to those very high levels of mite allergens.

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